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Chronic lymphocytic leukemia (CLL) is the most common form of lymphoid malignancy in Western countries[1]. Recent multi-stage genome-wide association studies (GWAS) have shown that part of the eight-fold increased risk of CLL seen in first-degree relatives of patients can be ascribed to the co-inheritance of multiple low-risk variants.[2–6]

Current projections for the number of independent regions harbouring common variants that are associated with CLL suggest that additional risk loci conferring modest effects should be identified by the expansion of discovery GWAS data sets.[2]

In this study, we have made use of a meta-analysis of GWAS data, followed by validation in multiple independent case–control series, to identify a novel susceptibility locus for CLL at 12q24.13.

The discovery phase comprised two previously described GWAS conducted in the United Kingdom[2,5] (see Supplementary Methods). UK-GWAS-1; 517 CLL cases (155 enriched for genetic susceptibility by virtue of family history) genotyped using Illumina HumanCNV370-Duo BeadChips[5] and 2698 controls from the Wellcome Trust Case Control Consortium 2 (WTCCC2) 1958 Birth cohort, typed using Hap1.2M-Duo Custom array.[7] UK-GWAS-2; 1271 CLL cases genotyped using the Illumina Omni Express BeadChip and 2501 UK Blood Service Donor controls typed using Hap1.2M-Duo Custom arrays.[2] To harmonise GWAS data sets we recovered untyped genotypes by imputation using IMPUTEv2 with 1000genomes as a reference (phase 1 integrated variant set (b37) from March 2012) (Supplementary Methods). Genomic control lambda values for UK-GWAS1 and UK-GWAS2 were 1.04 and 1.05, respectively, thereby excluding significant differential genotyping or cryptic population substructure.[2]

Post quality control the two GWAS provided data on 1739 cases and 5199 controls. In a meta-analysis we identified 156 common

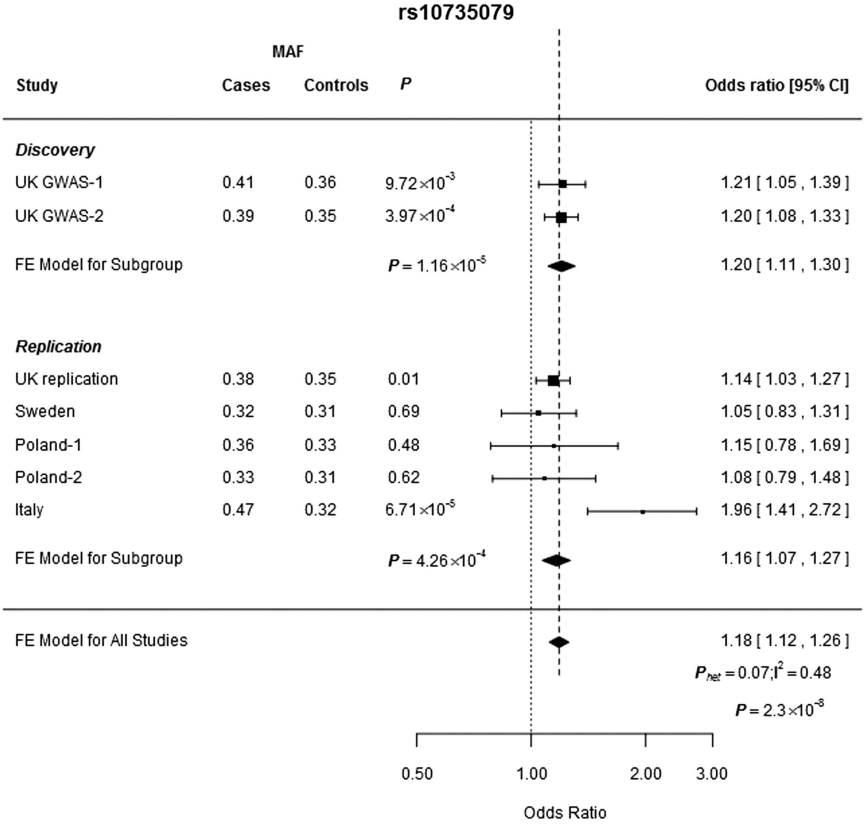

Figure 1. Forest plot of the ORs for the association between CLL and rs10735079. Studies were weighted according to the inverse of the variance of the log of the OR calculated by unconditional logistic regression. Horizontal lines: 95% CI. Box: OR point estimate; box area is proportional to the weight of the study. Diamond (and broken line): overall summary estimate, with CI given by its width. Unbroken vertical line: null value (OR = 1.0). FE, fixed effects; MAF, minor allele frequency.

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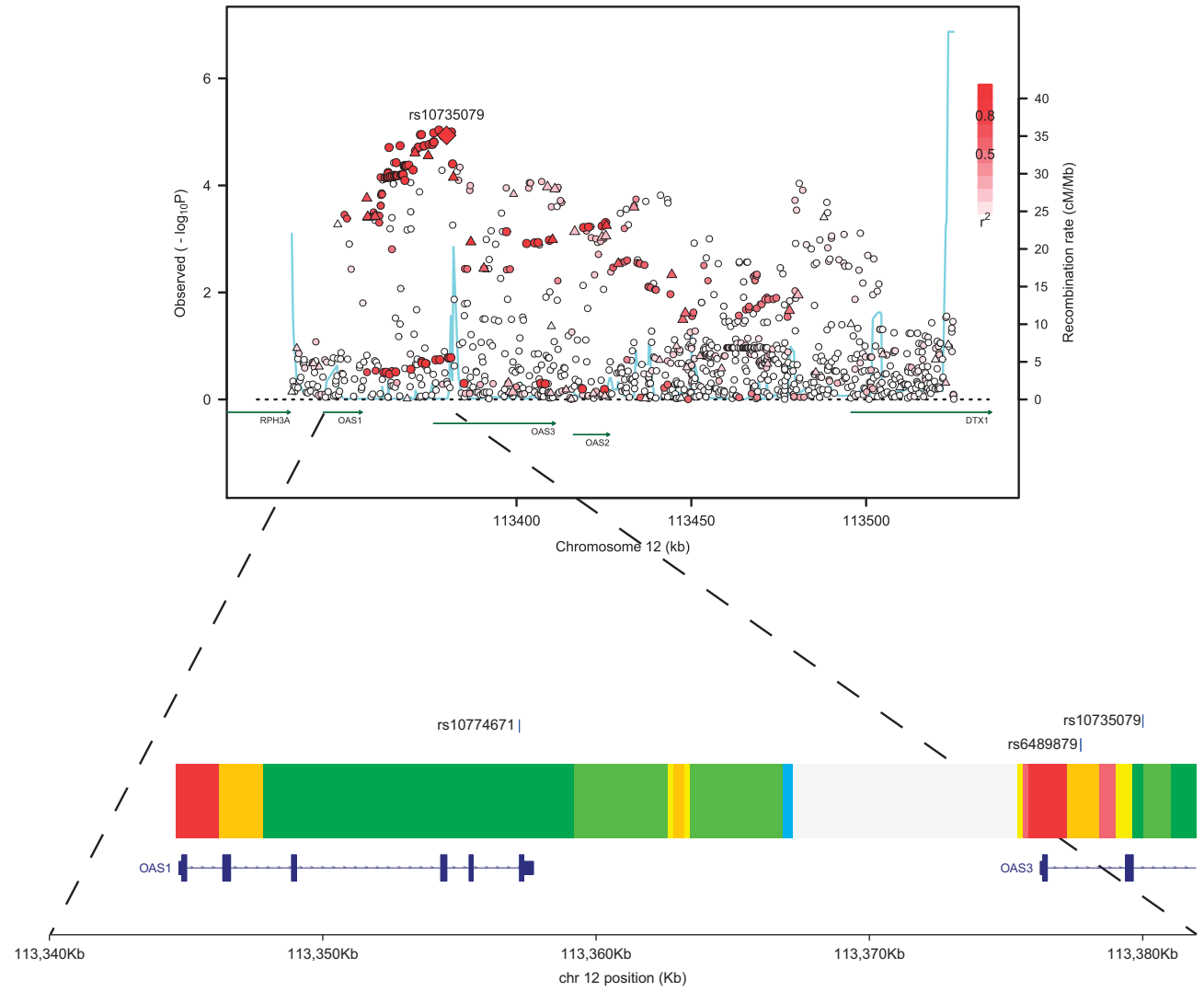

Figure 2. Regional plot of association results, recombination rates and chromatin state segmentation track for 12q24.13 susceptibility locus. Association results of both genotyped (triangles) and imputed (circles) SNPs in the GWAS samples and recombination rates. $-\log_{10}$ *P*-values (*y* axis) of the SNPs are shown according to their chromosomal positions (*x* axis). rs10735079 is shown as a large diamond and is labelled by its rsID. Colour intensity of each symbol reflects the extent of LD with the top genotyped SNP; white ($r^2 = 0$) through to dark red ($r^2 = 1.0$) Genetic recombination rates, estimated using HapMap Utah residents of Western and Northern European ancestry (CEU) samples, are shown with a light blue line. Physical positions are based on NCBI Build 37 of the human genome. Also shown are the relative positions of genes and transcripts mapping to the region of association. Genes have been redrawn to show the relative positions; therefore, maps are not to physical scale. The lower panel shows the chromatin state segmentation track (ChromHMM) for LCL data derived from the ENCODE project and the positions of SNPs of interest (produced using visPIG-Visual Plotting Interface for Genetics).

SNPs (minor allele frequency > 0.01), typed in either UK GWAS-1 or 2, that showed good evidence of an association (ie $P < 1.5 \times 10^{-4}$) and did not map to any of the 30 loci that have previously been associated with CLL risk.[2]

Seven SNPs chosen on the basis of strength of association and/or biological plausibility of the annotated gene (that is, a role in B-cell or cancer biology) were genotyped in the UK replication series (Supplementary Table 1), which comprised 1195 CLL cases ascertained from an ongoing national study being conducted by the Institute of Cancer Research and 2568 controls ascertained through the National Study of Colorectal Cancer[8] (Supplementary Methods).

Two SNPs, rs10735079 and rs17512800, provided further evidence for an association with CLL risk (ie $P < 0.05$) and these two SNPs were taken forward for genotyping in a further replication series from Sweden, which comprised 347 CLL cases and 342 controls (Supplementary Table 1). This case control analysis provided additional evidence for an association between rs10735079 and CLL risk. Subsequently we genotyped rs10735079 in three further case–control series, Poland-1 (105 cases, 101 controls), Poland-2 (176 cases, 209 controls) and Italy (186 cases, 155 controls) (Supplementary Methods). In the combined analysis of all series the association between rs10735079 and CLL attained genome-wide significance (combined OR per allele = 1.18, 95% CI:1.12–1.26, $P = 2.34 \times 10^{-8}$) (Figure 1). The association was not restricted to *IGHV* mutation and showed no relationship with either sex or age (Supplementary Table 2).

rs10735079 maps to intron 2 of the 2′-5′-oligoadenylate synthetase 3 (*OAS3*) gene, one of three *OAS* genes clustering at 12q24.13 (Figure 2), and is in LD ($r^2 = 0.87$) with the splice acceptor variant of *OAS1*, rs10774671, which mediates alternative splicing of *OAS1* transcription and affects enzymatic activity.[9]

Although attractive as the basis of the 12q24.13 association the association with CLL is stronger for rs10735079 than rs10774671 ($P = 1.16 \times 10^{-5}$ and 1.74×10^{-4}, respectively; Supplementary Table 3).

The significant dose relationship between rs10735079 genotype and *OAS3* expression in blood, with the risk allele being associated with reduced levels of mRNA ($P = 5.4 \times 10^{-29}$; Supplementary Table 4), supports a role for rs10735079 genotype mediating its effect on CLL through differential *OAS3* expression rather than impacting on *OAS1*.

Although rs10735079 is not predicted to lie in an active promoter or strong enhancer element, the correlated SNP rs6489879 ($r^2 = 0.99$) that maps to intron 1 of *OAS3* resides in a region predicted to be a strong enhancer in lymphoblastoid GM12878 cells and to be involved in binding of a number of transcription factors including IRF4 (interferon regulatory factor-4), a lymphocyte-specific transcription factor (Figure 2; Supplementary Table 3).

OAS is induced by interferon in response to viral infection activating 2-5A-dependent RNase L degradation of viral RNA[10] and variation in OAS genes has been reported to be a determinant of viral susceptibility.[9,11–13] Given the possible role of viral response in the pathogenesis of CLL, although speculative, it is therefore possible that genetic variation in *OAS3* influences risk of developing CLL through differing response to antigenic challenge. Moreover, *OAS3* is a B-cell receptor (BCR) signature gene.[14] Intriguingly as variation in the BCR genes *IRF4* (ref. 5), *BCL2* (ref. 3) and *HLA-DQA1* (ref. 15) has previously been implicated by GWAS as determinants of CLL risk this suggests a common aetiological pathway through differential BCR-activation.

Although further functional studies are required to fully elucidate the biological basis of the 12q24.13 association, our finding brings the total number of risk loci identified for CLL thus far to 31 and provides additional support for the role of inherited genetic factors in the aetiology of CLL.

URLS

Blood eQTL browser: http://genenetwork.nl/bloodeqtlbrowser/

PLINK: http://pngu.mgh.harvard.edu/~purcell/plink/

Illumina: http://www.illumina.com/

Kaspar: http://www.lgcgenomics.com/genotyping/kasp-genotyping-chemistry/

SNAP: http://www.broadinstitute.org/

Haploreg: http://www.broadinstitute.org/mammals/haploreg/haploreg.php

visPIG-Visual Plotting Interface for Genetics: http://vispig.icr.ac.uk/

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

RSH obtained financial support, designed the project and provided overall project management; GPS and RSH drafted the manuscript; GPS and HES performed project management and supervised genotyping; JMA obtained financial support and supervised genotyping; GPS and MCDB performed bioinformatic and statistical analyses; GPS and AH performed genotyping; YW and MCDB performed imputation analysis; MJSD, NJS, HM, LM, SD, LK, IF, DW, KJ, GJ, KES, SJ, AM, CD, AGH, TM, GHJ, GS, RJH, ARP, DJA, JRB, GP, CP, CF, RR, DC and JMA acquired samples for the replication cohorts.

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Classifying ultra-high risk smoldering myeloma

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Multiple myeloma (MM) always evolves from a precursor state, either monoclonal gammopathy of undetermined significance or smoldering MM (SMM).[1,2] These precursor states are defined by the absence of MM-related organ damage ('CRAB' features: hypercalcemia, renal failure, anemia and bone disease).[3] SMM has a highly variable clinical behavior, where some patients have an imminent risk of developing CRAB features and others have a substantially lower risk.[4,5] A recent study has demonstrated that the treatment of high-risk SMM patients with lenalidomide and dexamethasone resulted in fewer CRAB events and improved the overall survival.[6] This provocative study suggests that treatment of high-risk SMM may be beneficial. However, identifying patients at ultra-high risk of progression ($>80\%$ at 2 years) has proven difficult.[7] Both the Mayo Clinic and PETHEMA models fail to define such a high-risk group.[4,5,8] Furthermore, the PETHEMA model employs a flow cytometry technique, which is not widely available or validated. Genomic approaches have also failed to identify ultra-high risk groups.[9–11] Notably, a recent small study highlighted a fair degree of discordance among these models in SMM.[12] Bone marrow plasma cell (BMPC) infiltration of $\geqslant 60\%$ in SMM patients is associated with a 95% risk of developing MM within 2 years.[13] A major limitation remains that these models have not been validated in other populations.

Thus, we sought to identify novel biomarkers and validate existing models. We performed a retrospective study of patients with SMM seen at the University of Pennsylvania from 2008–2012. Eligibility was based on ICD-9 codes for MM (203.00–203.02), monoclonal gammopathy (273.1, 357.89) and plasma cell neoplasm (238.6). SMM was diagnosed based on the International Myeloma Working Group (IMWG) criteria[3]. Progression to active MM was based on clinician judgment that MM-related organ or tissue impairment had occurred or the initiation of MM therapy. The Institutional Review Board of the University of Pennsylvania approved this study.

Time to progression (TTP) to MM was defined as the time from SMM diagnosis to first documentation of progression to MM. Patients who had not progressed to MM at the time of statistical analysis were censored. The median TTP rate and 2-year progression rate were estimated by the Kaplan–Meier method. Association between TTP and each clinical factor was assessed by Cox regression analysis. Classification and regression tree (CART) analysis was employed to identify optimal cut points to dichotomize continuous variables that were candidates for the models. Multivariable Cox modeling was then performed using a backward elimination selection strategy. All analyses were performed in either SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) or STATA 13 (StataCorp, College Station, TX, USA). *P*-values <0.05 were considered statistically significant.

We screened 2777 patients and identified 135 with SMM. Nine patients with light-chain only SMM were excluded from the primary analysis. The median age was 62 years; 55% male; 78% Caucasian, 19% African American and 3% other races. Median follow-up was 4 years. Two patients died from non-myeloma related causes of death and were censored. The median TTP was 6.8 years with a two-year rate of progression of 28%.

We constructed a risk stratification model. CART analysis identified optimal cut points for three risk factors: (BMPC) $\geqslant 40\%$ (hazard ratio (HR) 2.72, 95% condifence interval (CI) = 1.28–5.77, $P<0.009$), serum-free light-chain ratio (sFLCR) $\geqslant 50$ (HR 4.57, 95% CI = 1.99–10.50, $P<0.001$) and albumin $\leqslant 3.5$ (HR 3.38, 95% CI = 1.57–7.27, $P<0.001$). M-protein concentration was excluded from the model owing to strong colinearity with sFLCR ($P<0.001$). Patients with zero risk factors were classified as low risk, patients with one risk factor were classified as intermediate risk and patients with two or three risk factors were classified as high risk.

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